

# THE METAMORPHOSIS OF PARTIAL LARVAE OF PERONELLA JAPONICA MORTENSEN,<sup>1</sup> A SAND DOLLAR<sup>2</sup>

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Following the discovery by Hans Driesch (1891), that in sea urchins an isolated blastomere of the two-cell stage can develop into an harmonic larva, much work has been done on this subject. However, since the majority of experiments in the past have been concerned with the developmental capacity only as far as the pluteus stage, emphasis in the present investigation was placed on the aspect of metamorphosing capacity. The inadequacy of past experiments is due to the difficulty of rearing plutei to metamorphosis in the majority of sea urchin species, which, fortunately, can be obviated in the sand dollar *Peronella japonica* Mortensen. In this species metamorphosis is completed in the course of three or four days.

*Peronella* is found in great numbers on the sandy bottom of a shallow lagoon in the neighborhood of the Misaki Marine Biological Station, and the breeding season extends from the latter part of June to September.

The main developmental features of this animal have been worked out by Mortensen (1921), but some new points were noticed by the authors. In the present paper, the results obtained by operative experiments, and points regarding normal development which have a direct concern with the description of the experiments will be reported.

## I. DEVELOPMENT OF THE WHOLE LARVA

*Egg.* The egg, about 300  $\mu$  in diameter, is heavily laden with yolk and opaquely pink. The color is imparted by a rosy pigment which is associated with doubly refractive crystals.

Elevation and hardening of the fertilization membrane are very slow, so that the membrane can be removed until 10 minutes after insemination. Since the hyaline layer is extremely delicate and remains sticky, the denuded eggs adhere to the bottom of the container, and after cleavage, the blastomeres arrange themselves in a plane, suggesting sea urchin blastomeres in calcium-low sea water. Later, however, the larva rounds up and gives rise to a typically spherical blastula.

*Micromeres.* Frequently, in this species, at the fourth cleavage all sixteen cells are of equal size. In such cases, the micromeres are formed in the succeeding cleavage. The micromeres, are, as a rule, relatively large in comparison with those of other echinoderms. Toward the end of the breeding season, however, there is an increasing tendency to produce smaller micromeres. In eggs which are considered to be more or less overripe, as early as the eight-cell stage, the vegetal four

<sup>1</sup>Dr. F. Uchinomi called the authors' attention to the fact that *Peronella lesucuri* has recently been changed to *Peronella japonica* Mortensen by Mortensen (1948), for which the authors' thanks are due.

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blastomeres are smaller than the animal blastomeres; and in the sixteen-cell stage, still smaller micromeres are formed.

*Blastula.* At about five hours after fertilization, one or two grooves are noticeable in the blastula, coinciding with the cleavage-furrows of the two- or four-cell stages. However, these grooves gradually flatten out as the blastocoel expands, and the embryo again becomes spherical. This is somewhat similar to the condition which is found in the development of *Astropecten aranciacus* (Hörstadius, 1939a).

At about seven hours after fertilization, the blastula acquires cilia and begins to rotate within the fertilization membrane. From this time on, primary mesenchyme cells migrate inward and disperse in the blastocoel, so that the embryo takes on an opaque appearance. At nine hours, the embryo breaks through the membrane; it is now elongated, with a truncated posterior end having an accumulation of primary mesenchyme cells along the vegetal wall, so that the larva looks somewhat like the

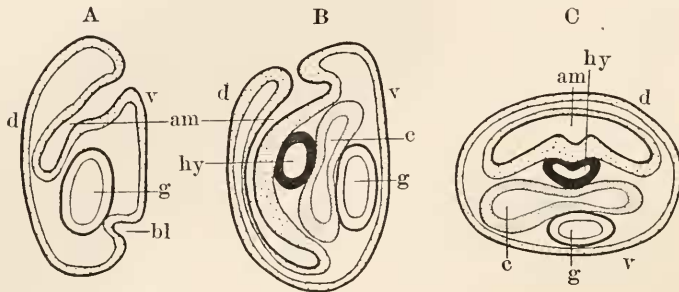


FIGURE 1. Diagram of section of successive stages showing the position of amniotic cavity and hydrocoel. A, B, longitudinal sagittal section. A, late gastrula (18 hours); B, early pluteus (26 hours), amniotic opening has shifted toward the dorsal side and blastopore has closed. C, transverse section, early pluteus (26 hours). am, amniotic cavity. hy, hydrocoel. c, coelom. g, gut. bl, blastopore. d, dorsal side. v, ventral side.

gastrula of regular sea urchins. The apical tuft does not usually differentiate, and even when it can be recognized, the cilia are fewer and less conspicuous than in other forms.

*Gastrula with the amniotic invagination.* Gastrular invagination begins at about the twelfth hour. Within a few hours, another ingrowth of the ectoderm appears in the center of the flattened oral field (Fig. 7 D, W) and develops into a stomodaeum-like invagination, but that it is not a true stomodaeum is shown by its subsequent failure to unite with the archenteron. Instead, its sac-shaped prolongation extends along the dorsal side of the entoderm and forms a cavity (Fig. 1, A). Mortensen correctly identified this as an amniotic cavity. No mouth opens, and the blastopore closes sooner or later, so that the archenteron remains as a free blind sac within the body, without forming any functional digestive tract. This obviates the necessity for feeding the larvae and greatly facilitates their culture.

*Pluteus.* At about twenty hours after fertilization, the fully formed pluteus typically has only two post-oral arms, equal in length to the rest of the body. The number of arms may vary, however, from none to four, without apparently causing any essential difference in the later development, since all such larvae are able to

complete metamorphosis. At this stage, the hydrocoel begins to differentiate from the coelomic sacs, which lie close to the ventral side of the body. The hydrocoel is usually derived from the left, but occasionally from the right, coelom; in either case, it is formed in a nearly median position (Fig. 1, B, C). This location of the hydrocoel, together with the unusual median position of the amniotic invagination, forms a

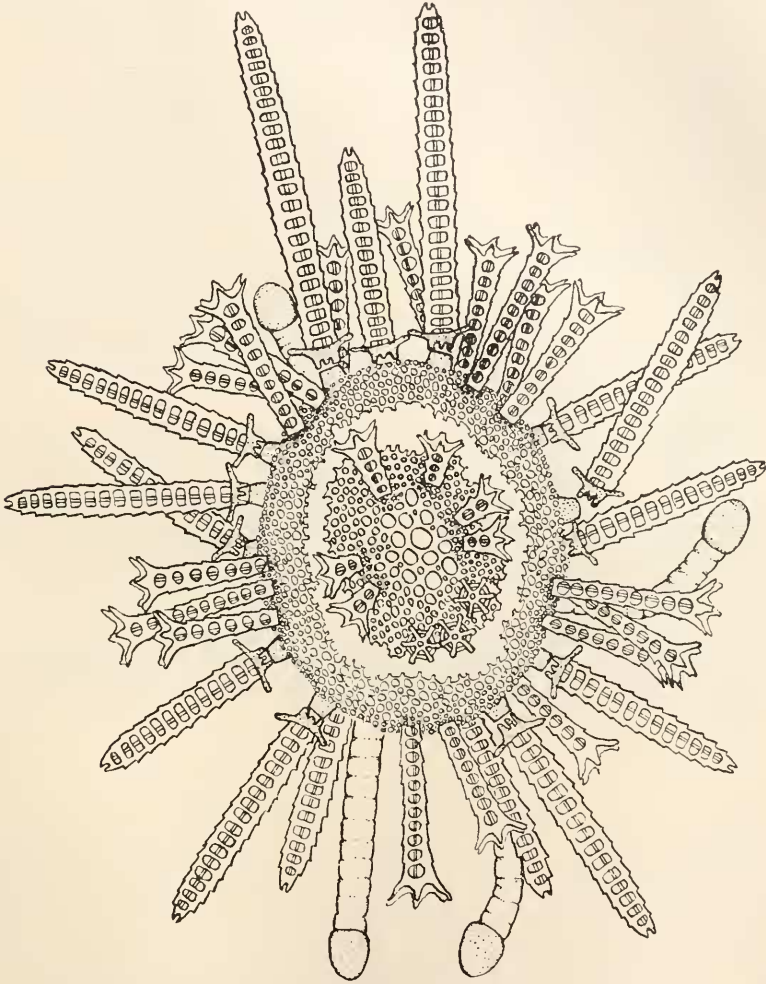


FIGURE 2. Control whole imago of *Peronella japonica* Mortensen, 4 days after metamorphosis. Aboral view.  $\times 160$ .

striking contrast to the location of the corresponding structures in other echinoderms. After the enlargement of the hydrocoel, five lobes are pushed out, arranged in a bilaterally symmetrical fashion with regard to the median plane of the pluteus. At about fifty hours after fertilization, the amniotic cavity constitutes a large part of the pluteus and its inner wall is covered with well-developed adult spines. The pluteus sinks to the bottom at this stage.

*Pigment.* Simultaneously with the formation of adult spines and plates, clypeastroid pigment (green) is deposited in the echinus rudiment and is gradually concentrated as development advances. The pluteus, as a whole, is greenish at this stage, but if it dies, a splendid green color pervades the entire body. Although this pigment is green in an alkaline fluid, it gradually loses its color completely as the medium is acidified. The green pigment is also found in the mesenchyme cells of the late pluteus of *Astriclypeus manni* and *Clypeaster japonicus*, especially clearly in the echinus rudiment. If the test of an adult *Peronella*, *Astriclypeus* or *Clypeaster* is injured, the same green color appears on the test integument.

*Metamorphosis.* Among relatively fast-growing larvae of *Peronella*, metamorphosis sets in at about sixty hours after fertilization with the protrusion from the amniotic cavity of rudimentary spines and tube-feet. Since the dorsal wall of the body and the amnion are very thin at this time, they are occasionally broken through by the protruding spines. On the contrary, slowly growing larvae, in spite of the fact that they have the typical pluteus form, may fail to metamorphose, or finally succeed after a delay of a week or more. When metamorphosis fails completely in such retarded larvae, they eventually become edematous. In such a case, the already differentiated spines degenerate and disperse within the body as fine spicules. It is interesting that in such degenerating larvae, the above-mentioned clypeastroid pigment cannot be recognized at all.

Usually, metamorphosis is completed within seventy or eighty hours. Concerning the external features of the adult, Onoda's report is available (1937). The mouth opening, masticatory apparatus, typical spines and six-rayed spines and tube-feet can be recognized a few days after metamorphosis (Fig. 2). The young sand dollars survive for about ten days without being fed, and can even increase the number of spines and tube-feet.

The most unusual feature in the development of this animal is certainly the rapid rate of development as compared with other related forms. It can be said that the pluteus stage is only a phantom, so to speak, and the larva is preparing for metamorphosis from the start.

## II. DEVELOPMENT OF PARTIAL LARVAE

The fertilization membranes were removed immediately after fertilization by squirting the eggs through a slender pipette, and the blastomeres were separated in sea water by a fine glass needle. In this form the hyaline layer is so delicate that it can easily be cut, even in a calcium-containing medium. Pairs of half-larvae or quartettes of quarter-larvae were kept in separate glass containers in 5 cc. of sea water. The plane of section at each stage is shown diagrammatically in Figure 3, and the results of the experiments are summarized in Table I and indicated in Figure 4.

- (1) Isolated blastomeres of the two-cell stage; *equi-* and *toti-potent* regarding *metamorphosing capacity*.

Blastomeres isolated in this stage show partial cleavage as regards the number of meso-, macro- and micromeres, and form more or less open half-blastulae, which, however, soon round up and close, and gastrular invagination occurs at the vegetal

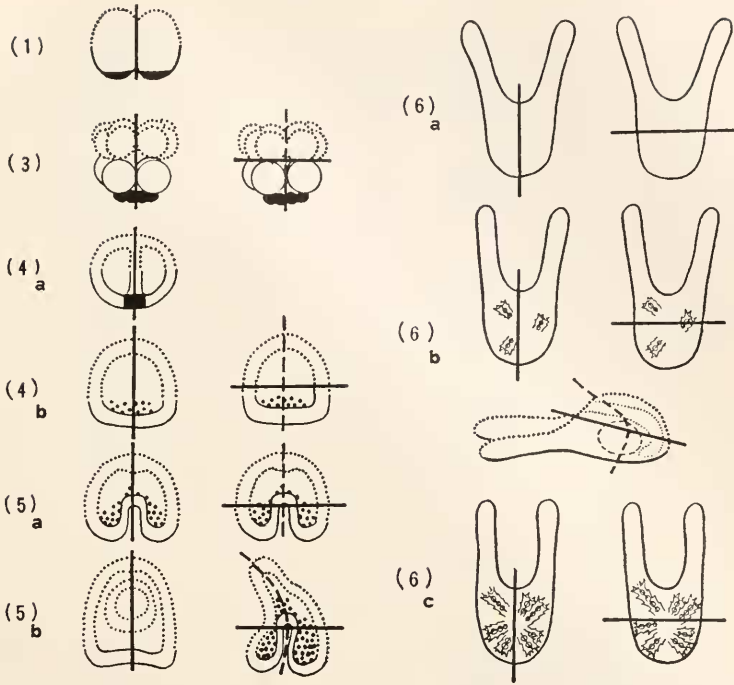


FIGURE 3. Diagram indicating plane of section at each stage. Dotted line, animal elements; fine continuous line, vegetal elements. Thick straight line, plane of sections; broken line, original egg-axis. Stages of operation numbered as follows: (1), 2-cell. (3), 16-cell. (4)a, grooved blastula. (4)b, blastula with primary mesenchyme cells. (5)a, gastrula with archenteron and two small triradiate spicules. (5)b, late gastrula with amniotic invagination. (6)a, early pluteus with two fully developed arms but without adult skeleton. (6)b, pluteus with some adult skeleton. (6)c, late pluteus with well developed adult skeleton.

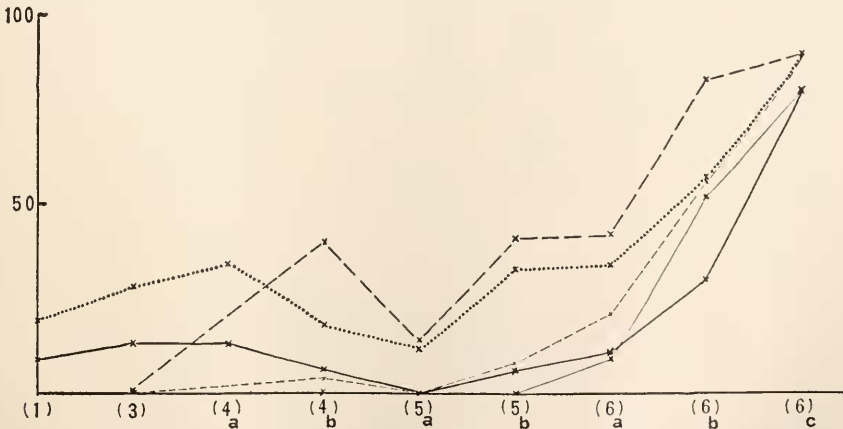


FIGURE 4. Percentage of metamorphosis of separated halves of *Peronella japonica* Mortensen, Dotted line, meridional halves. Thick continuous line, meridional pairs. Thick broken line, vegetal (posterior) halves. Fine broken line, animal (anterior) halves. Fine continuous line, equatorial pairs.

TABLE I  
*Number and percentage of metamorphosed half larvae*  
 Meridional halves

Stage of operation	(1) Two-cell stage	(2) Sixteen-cell stage	(4)a Grooved blastula stage	(4)b Blastula stage with mesenchyme cells	(5)a Gastrula stage	(5)b Late gastrula stage with amniotic invagination	(6)a Early pluteus stage with no adult skeleton	(6)b Pluteus stage with some adult skeleton	(6)c Late pluteus stage with well-developed adult skeleton
Hours after fertilization	1	2½ - 3	5 - 7	7 - 9	11 - 15	15 - 17	19 - 25	45 - 55	70 - 90
Number of operated larvae	236	45	32	53	22	15	28	27	20
Number and percentage of metamorphosed pairs	22 9%	6 13%	4 13%	3 6%	0 0%	1 6%	3 11%	8 30%	16 80%
Number of metamorphosed single halves	46	13	14	13	5	8	13	15	4
Number and percentage of metamorphosed meridional halves*	90 19%	25 28%	22 34%	19 18%	5 11%	10 33%	19 34%	31 57%	36 90%

TABLE 1—Continued  
Equatorial halves (anterior and posterior halves)

Stage of operation	(1) Two-cell stage	(3) Sixteen-cell stage	(4)a Grooved blastula stage	(4)b Blastula stage with mesenchyme cells	(5)a Gastrula stage	(5)b Late gastrula stage with amniotic invagination	(6)a Early pluteus stage with no adult skeleton	(6)b Pluteus stage with some adult skeleton	(6)c Late pluteus stage with well-developed adult skeleton
Number of operated larvae		431		25	37	12	33	29	20
Number and percentage of metamorphosed pairs		0 0%		0 0%	0 0%	0 0%	3 9%	15 52%	16 80%
Number of metamorphosed animal (anterior) single halves		0		1	0	1	4	1	2
Number and percentage of metamorphosed animal (anterior) halves†		0 0%		1 4%	0 0%	1 8%	7 21%	16 56%	18 90%
Number of metamorphosed vegetal (posterior) single halves		4		10	5	5	11	9	2
Number and percentage of metamorphosed vegetal (posterior) halves‡		4 1%		10 40%	5 14%	5 41%	14 42%	24 83%	18 90%

\* Single halves + 2 members of each pair.

† Single animal halves + animal half of pairs.

‡ Single vegetal halves + vegetal half of pairs.

side. Such operation at the two-cell stage does not destroy the basic arm-forming capacity of the larvae, since the resultant plutei usually have two arms, and very rarely three- or four-armed half-plutei are encountered. However, the general tendency is toward a reduction in the number of arms as the result of the operation ;

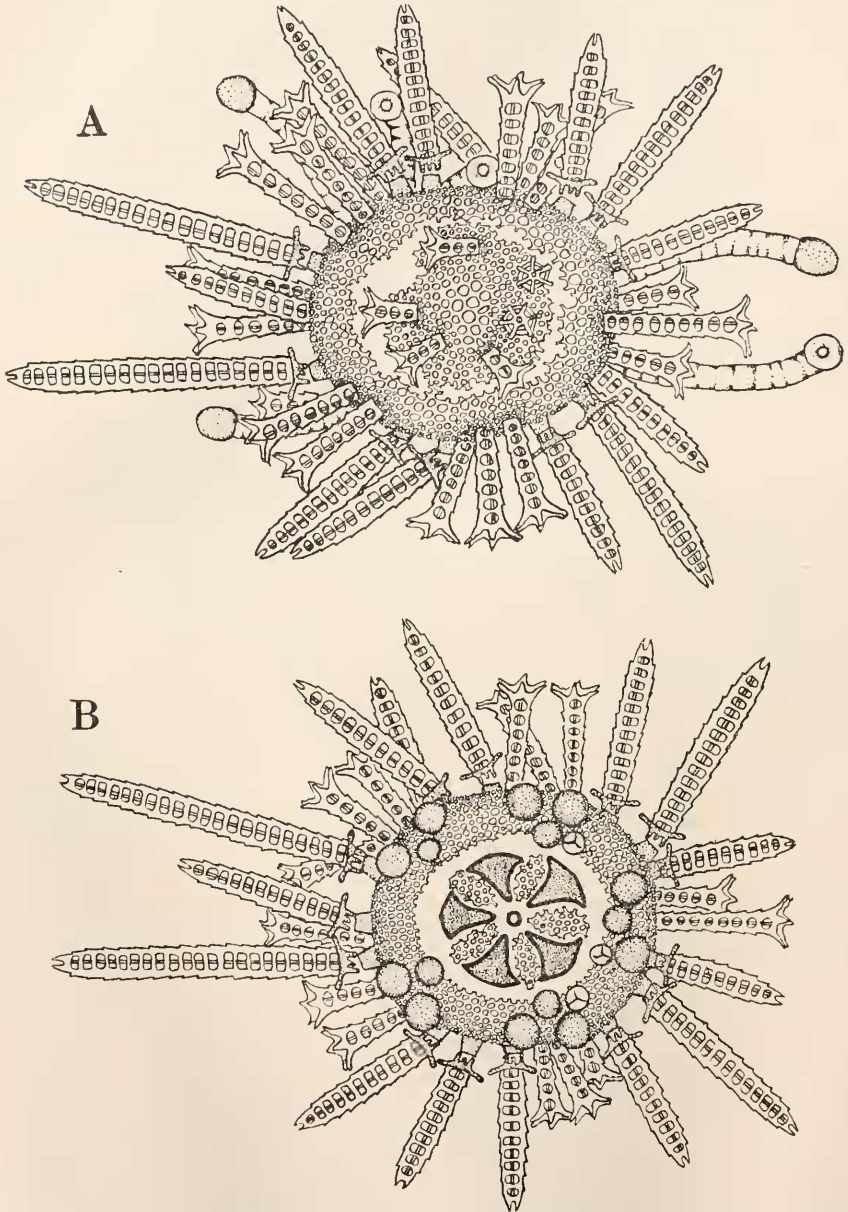


FIGURE 5. Pair of half-imagos derived from single blastomeres of the two-cell stage, 4 days after metamorphosis. A, aboral view. B, oral view. They do not show any difference from whole imagos except for their small size.  $\times 160$ .



*i.e.*, the percentage of one-armed plutei is greater among the half-larvae than among the unoperated controls. Even in this case, however, it does not mean that each of the pairs has a right and left arm, respectively. At any rate, development of the arms, as in the case of the whole larva, shows no connection with the capacity of the half-larva for metamorphosis.

The young half sand dollar does not show any difference from the whole sand dollar except that it is dwarf, the two members of the pair being completely equal in all points (Figs. 2, 5). There is not even much delay in development as a result of the operation.

(2) Isolated blastomeres of the four-cell stage: *equi- and toti-potent regarding metamorphosing capacity.*

The results of the operation are as follows:

Operated 4-cell stages .....	207
All members of the quartettes metamorphosed .....	2 sets
Three members of the quartettes metamorphosed .....	6 sets
Two members of the quartettes metamorphosed .....	24 sets
One member of the quartettes metamorphosed .....	65 singles

In contrast to the relative frequency with which both members of a pair of half-larvae completed metamorphosis, all four quarter-larvae derived from isolation in the four-cell stage were rarely able to metamorphose. The probable reasons for this are presented below.

The development of quarter-larvae is much like that of the half-larvae described above, except for their smaller size. However, quarter-plutei usually have only one arm or none at all. The number of arms has, again, no essential meaning for metamorphosis.

The physiological condition of the quarter-imagos seems to be inferior to that of the half-imagos, since the metamorphosis of the former is delayed as compared with that of the latter, and many of the former die soon after metamorphosis. Even when they survive for a while, they do not usually show any sign of growth and eventually become edematous. But a point to be stressed is that even in such quarter-imagos, no part of the body is missing.

There were, altogether, 8 instances in which three or four members of a quartette metamorphosed. In these cases, not only was the general developmental condition very poor, but the respective rates of growth and degrees of differentiation were extremely variable (Fig. 6). On the contrary, there were 89 cases in which one or two members of a quartette succeeded in metamorphosing. These imagos appeared to be much better developed than those of the 8 cases in which three or four survived, occasionally even reaching a state comparable to that of half-imagos. This may probably mean that  $\frac{1}{4}$  of the protoplasm of a single egg is about the minimum of material sufficient to permit metamorphosis; and further, that when blastomeres of the 4-cell stage do not share the egg protoplasm strictly equally, the smaller ones fall below the viable level. Harvey (1940) reported the similar fact that although isolated quartettes from a single egg of *Arbacia punctulata* may all develop into perfect dwarf plutei, there is considerable variation in size among them.

(3) Half-larvae of the 16-cell stage: *Meridional halves: equi- and toti-potent with respect to metamorphosis.*

Of 45 pairs of half-larvae obtained by meridional section of the 16-cell stage, 6 pairs and 13 singles completed metamorphosis. Early half-imagos resulting from this operation are indistinguishable from those derived from the 2-cell stage.

*Equatorial halves: animal half fails to metamorphose; vegetal half shows weak capacity for metamorphosis, always followed by immediate death.*

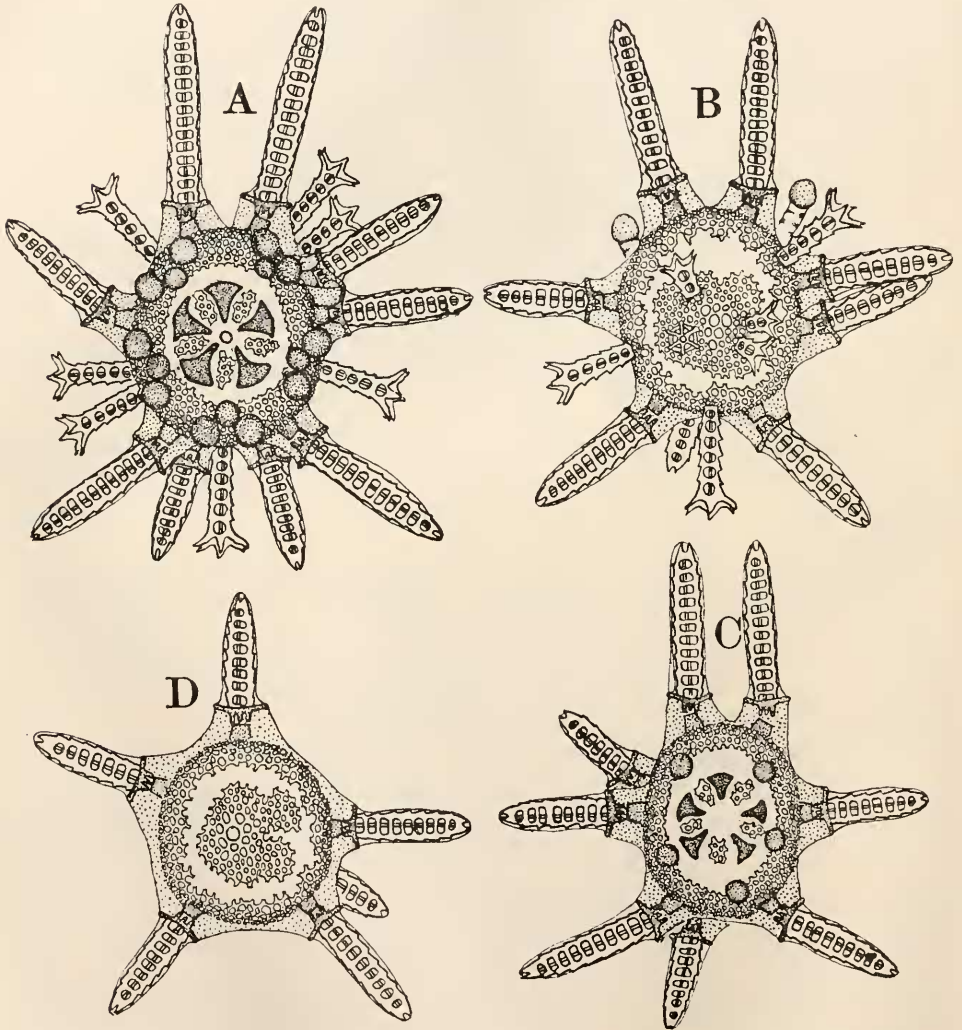


FIGURE 6. Quartettes derived from single blastomeres of the four-cell stage, 4 days after metamorphosis. A, C, oral view. B, D, aboral view. Their developmental condition is very poor as compared with half-imagos, and further, there is a lack of uniformity in their respective rates of growth.  $\times 160$ .

It is rather difficult to find differences in the mode of development of the animal and vegetal halves before the appearance of the primary mesenchyme cells, although the vegetal half tends to round up somewhat earlier than the animal half. Simultaneously with the migration of the primary mesenchyme cells, however, a distinction between the two halves suddenly appears. The vegetal half becomes opaque because of the presence of the mesenchyme cells in the blastocoel, while the animal half remains transparent (Fig. 7, A). Another striking difference between the two regions is that on dying, the animal half becomes a brilliant pink, while the vegetal half is turned green by the clypeastroid pigment. A detailed account of their respective courses of development follows.

*Animal half.* The blastula of the animal half gradually becomes flattened—usually in the dorso-ventral direction.<sup>3</sup> At about 12 hours after fertilization—in the control whole-larvae gastrular invagination begins at this time—a depression appears in the ventral field (Fig. 7, C, An). This is exactly the position at which amniotic invagination occurs in control whole-larvae a few hours later.<sup>4</sup> At this time, the animal half-larva has remarkably long cilia on almost the whole or a part of the surface, the wall of which is more or less thickened. A few hours later, larvae are frequently found with two, and occasionally with three depressions at once (Fig. 7, D, An II). Some larvae have separate cells in the blastocoel, but probably these are not mesenchyme cells, since they neither form spicules nor contain clypeastroid pigment. In other words, they do not show any differentiation. The situation is the same for amphibians (Ruud, 1925; Vintemberger, 1934, 1935).

Several hours later, the wall of the animal region rapidly increases in area and begins to wrinkle (Fig. 7, E, An). The wrinkled area spreads from the animal toward the vegetal side, so that these blastulae finally have the appearance of a mass of many small ciliated vesicles (Fig. 7, E, An), closely resembling the isolated and cultured *Triton* epidermis as described by Holtfreter (1933). Occasionally, separate small vesicles fall off from the main mass, and some of these swim around as small blastulae without showing any further development even after several days. As a rule, animal half-larvae have a tinge of pink, in contradistinction to the greenness of the mesenchyme cells, but upon death the pink color increases its brilliance and often pervades the entire body. Although a pink pigment exists originally in the unfertilized egg of *Peronella*, and, moreover, the epidermis of normal larvae has a slight tinge of the same color, their tones are not so deep as that of the dead animal half. Occasionally, such a pink color is also recognized in a part of a whole embryo which has died at the morula or the early blastula stage.

<sup>3</sup> The animal pole region of the gastrulae is indicated by a tuft of long cilia. On animal half-larvae after flattening, these long cilia are usually found at one pole of the long axis. Occasionally, however, cases are found in which one of the flattened sides bears somewhat longer cilia than the other surfaces (Fig. 7 B, An I).

<sup>4</sup> At the stage of formation of the amniotic invagination, not only the animal half- but also the control whole-larva becomes flat in the dorso-ventral direction (Fig. 7 D). It may be possible that the animal half which is released from the effect of vegetal elements flattens, and amniotic invagination occurs earlier than normally. Moreover, Hörstadius showed (1935, 1939b) that animal halves of *Paracentrotus* often develop into blastulae with stomodaea, and, as before mentioned, the amniotic invagination of *Peronella* bears a striking resemblance, morphologically, to the stomodaeum of the sea urchin larva. However, it is very difficult to judge whether the depression of the animal half-larva of *Peronella* corresponds to the amniotic invagination.

*Vegetal half.* The swimming vegetal blastula takes on a very dark appearance earlier than does the whole larva, since the blastular wall is extremely thick and the small blastocoel is filled with mesenchyme cells (Fig. 7, A, Ve). In a short time, the cells in the vegetal region begin to dissociate and fall out of the blastular wall (Fig. 7, B, Ve). The dissociated cells adhere to each other, forming a green mass on the outside of the vegetal wall (Fig. 7, C, Ve).

Some larvae soon cast off this mass of dissociated cells and develop into small gastrulae or plutei (Fig. 7, E, Ve), although a few exogastrulae with long, pro-

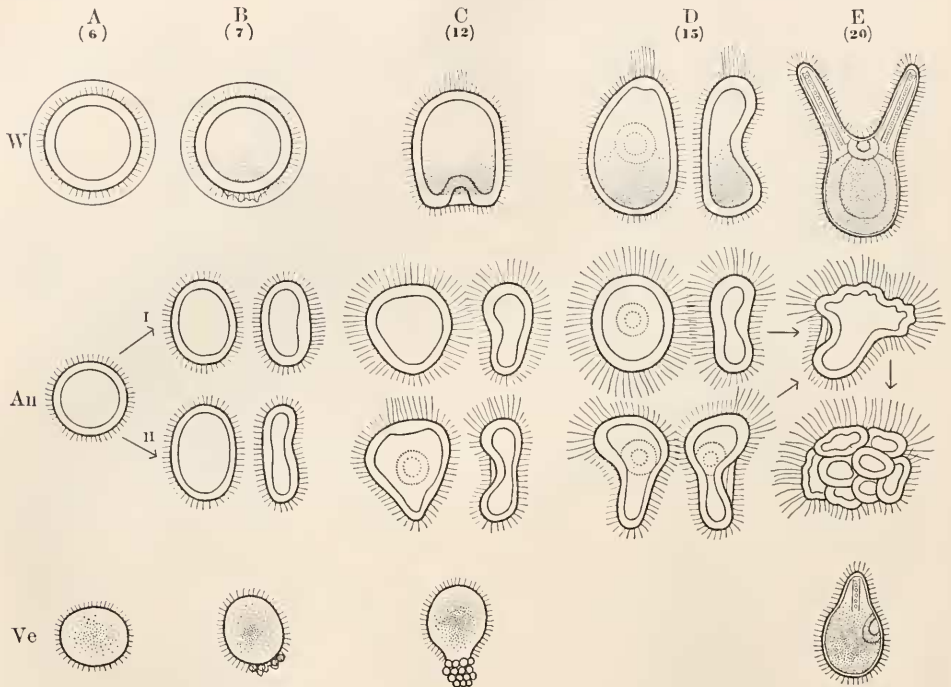


FIGURE 7. Comparison of development of equatorial half-larvae of 16-cell stage and control whole larvae of *Peronella japonica* Mortensen. Numbers of the upper row indicate hours after fertilization. W, control whole larvae. An, animal halves. Ve, vegetal halves. I and II, two types of animal half-larvae at each stage.

truding archenterons are also formed. Of 431 vegetal halves, 71 developed into plutei with or without arms. Four of these plutei succeeded in metamorphosing. However, such young imagos had only two or three spines on an extremely edematous body, and died immediately after metamorphosis.

On the other hand, when the mass of dissociated cells becomes larger than the larval body, which is rather frequently the case, the larvae adhere to the substratum with a part of the sticky cell mass and consequently are unable to swim in spite of their ciliary activity. Even if they succeed in freeing themselves from the cell mass, they can develop only into small blastulae filled with mesenchyme cells. This is no doubt because such larvae have lost too many cells to continue further development.

In either case, dead vegetal larvae exhibit a green color, although whole larvae of such a young stage never do so. It is quite an impressive contrast that the animal half turns a brilliant pink on death, while the vegetal half assumes a green color as it dies.

#### (4) Half-blastulae.

Larvae were bisected meridionally at the following two stages and equatorially at the second stage (see Fig. 3 (4) a, b):

- a. Grooved blastula (5 hours after fertilization).
- b. Late blastula with primary mesenchyme cells (7-9 hours after fertilization).

*Meridional halves: equi- and toti-potent with respect to metamorphosis.*

Grooved blastulae were bisected along the groove. The development proceeds in much the same way as in isolated blastomeres of the 2-cell stage. Since the grooves of the blastula coincide with the cleavage furrows of the two- or four-cell stage, the distribution of material in half-larvae of this kind is identical with that in half-larvae of the two-cell stage. Consequently, such operative results as were obtained are quite according to expectation. This experiment furthermore indicates that the regulative capacity in the meridional half has not at all decreased by the grooved blastula stage.

Halves separated at the late blastula stage go through metamorphosis only half as frequently as the previous stage and the physiological condition of the half-imagos is much poorer.

*Equatorial halves: metamorphosing capacity of the animal halves negligible, of vegetal halves strong.*

Among the equatorial halves, there was no instance in which both members of a pair succeeded in metamorphosing. Commonly, the animal half became a blastula with mesenchyme cells, and soon died without further development, except in one instance. However, this animal became extremely edematous and did not show typical differentiation and, moreover, its sister half (vegetal) developed only as far as the pluteus stage. It is likely that the plane of cutting might have been further toward the vegetal pole than usual.

In general the vegetal halves developed into plutei, usually having two arms, and many of them metamorphosed. The young imagos mostly showed typical differentiation and survived for several days after their metamorphosis.

#### (5) Half-gastrulae.

Larvae were operated on in the following two stages:

- a. Gastrula with archenteron and two small triradiate spicules (11-15 hours after fertilization).
- b. Late gastrula with amniotic invagination (15-17 hours after fertilization).

*Meridional halves: percentage of metamorphosis is minimal at the stage a, physiological condition poor, progressive decrease in regulative capacity.*

Results of operation through these stages show a general downward trend of regulative power. As for the degree of bodily differentiation, by the time the late gastrula stage is reached, many half-imagos show a reduced number of plates or tooth-rudiments or spines on the side of the operation.

The same is true with the number of arms: the later the stage of operation, the more larvae with one arm are produced until finally all of them become one-armed if operated on at the late gastrula stage.

However, from the standpoint of metamorphosing capacity, a rather unexpected result was obtained. It was found that the frequency of successful metamorphosis improves toward the end of the gastrula stage in spite of a continual loss of regulating power for bodily organization. Although it is recognized that operation during the invagination process seems to act more deleteriously than in later stages, the situation does not seem to be so simple, since in still later stages, such as the pluteus or imago stages, the percentage of metamorphosis keeps on improving despite more defective organization of the larvae.

*Equatorial halves: metamorphosing capacity of animal halves negligible, of vegetal halves rather strong.*

The development of the equatorial halves of the gastrulae is very much the same as that of operated blastulae. Animal halves can only reach the stage of blastulae with mesenchyme cells except one instance of metamorphosis in an extremely edematous larva. On the contrary, vegetal halves do metamorphose in much better condition and even survive several days after metamorphosis.

#### (6) Half-plutei.

Larvae were bisected at the following stages:

- a. Early pluteus with two fully developed arms, but without adult skeleton (19–25 hours after fertilization).
- b. Pluteus with some adult skeleton (45–55 hours after fertilization).
- c. Late pluteus with well-developed adult spines and plates (70–90 hours after fertilization).

The results are included in Table I and Figure 4.

*Meridional halves: equi- and toti-potent; capacity for metamorphosis stronger than that of half-blastulae and -gastrulae.*

Meridional half-larvae operated on at these stages had one arm and were not able to form another, but many of them metamorphosed, although many half-imagos became more or less edematous.

*Anterior and posterior halves: equi- and toti-potent with respect to metamorphosis.*

Anterior half-larvae apparently were not able to regenerate the posterior portion, and posterior half-larvae re-formed no arms, yet both halves metamorphosed. It is particularly worth mentioning that both anterior and posterior halves of a single larva are able to metamorphose.

In young imagos derived from anterior halves, the physiological condition seems to be inferior to that of those derived from meridional or posterior halves, since all the former became edematous and died without developing, while the latter survived longer and grew to some extent, although eventually they also became more or less edematous.

Usually, in any of these half-imagos, some lack of plates, spines or teeth is found on the side of the operation. This absence of parts in the half-imagos becomes more and more conspicuous as the stage of operation advances. However, the total number of the plates, spines and teeth found in the two halves from a bisected larva is always greater than that of the control whole imagos.

*Animal and vegetal halves: equi- and toti-potent in metamorphosing capacity.*

As indicated in Figure 3, (5)b and (6)b, the original egg-axis is bent at the pluteus stage, so that the anterior and posterior halves of the pluteus do not coincide with the animal and vegetal halves, respectively, of the blastula or gastrula, regarding the egg-axis. Therefore, in order to obtain half-larvae corresponding as nearly as possible in this respect to the animal and vegetal halves of the earlier stages, plutei were cut by a frontal section into front (animal) and rear (vegetal) parts (see Fig. 3, (6)b).

Of 15 plutei operated on, 7 pairs and 5 vegetal halves metamorphosed. These young sand dollars are much like those derived from meridional halves of plutei of the same age.

(7) Bisected imagos: both halves survive for several days and continue growth.

Young imagos were bisected immediately after metamorphosis. Of 10 imagos so treated, 7 pairs and 3 singles survived for several days after the operation and were able to increase the number of spines and tube-feet to some extent, although they could not regulate them to the typical numbers. It appears that physiological recovery in the bisected animal is relatively easy even after metamorphosis.

#### DISCUSSION

As is indicated in Figure 4, the metamorphosing capacities of both meridional and equatorial halves of *Peronella* larvae show a similar tendency to drop to a minimum at stage (5)a (gastrula stage). The regulative capacity, on the other hand, steadily decreases as the stage of operation advances. Such a relation is precisely that which would be expected from the experiments of previous workers (Jenkinson, 1911; Hörstadius, 1936, 1939b), so far as they go within the limit of the pluteus stage. However, the fact that all the curves of half-larval metamorphosing capacity rise steadily from the late gastrula stage on, seems to be of considerable significance. Especially animal halves, which were found to be almost lacking in metamorphosing capacity before the gastrula stage, acquire the capacity in the pluteus stage.

The interpretation of these curves is very difficult. However, several suppositions with regard to each point will be presented, although no final conclusion can be reached at this time.

The percentage of half-larvae operated on at the two-cell stage which are able to metamorphose is unexpectedly low. This is probably due to the fact that there is no way for the investigator to reject individuals with low viability at such an early stage, although this elimination is automatically realized when older larvae are used. If the selection of larvae with high viability were possible, a higher metamorphosing capacity than that which appears in the present results would undoubtedly be demonstrated.

When the four blastomeres of the four-cell stage are separated, all four quarter-larvae metamorphosed. This is a further amplification of Hörstadius' well known work. Corresponding experiments on *Amphibia* by Ruud (1925) indicate that the situation differs slightly in these forms, and only blastomeres carrying the future site of the organizer are totipotent. For mammals the data are lacking, except that Seidel (1952) succeeded in obtaining a perfect rabbit from one blastomere of a two-cell stage which was implanted in another female in the right physiological condition.

Of equatorial halves separated at the sixteen-cell stage, the animal halves developed only to the blastula stage, while the vegetal halves, generally speaking, were able to metamorphose. However, for some reason not yet understood, only a few of such vegetal halves were able to metamorphose. On the other hand, relatively many vegetal halves of blastulae or gastrulae were able to metamorphose, as compared with vegetal halves of the sixteen-cell stage. A possible explanation might be that since the ectoderm overgrows toward the vegetal pole as the larvae develop (see Fig. 3), the vegetal halves of the swimming stages contain a larger amount of ectoderm than similar halves of early stages, so that the balance between animal and vegetal elements in later stages will approach more closely to the normal than in earlier stages.

Although the direction of the egg-axis shows a clear correlation with the presence and absence of metamorphosing capacity through the gastrula stage, this is lacking in the pluteus stage, as evidenced by the fact that animal and vegetal half-larvae are equally able to metamorphose, and produce imagos resembling those developing from meridional halves of the same stage. This result indicates that some other factor has superseded in importance the original animal-vegetal relation by the time the pluteus stage is reached.

When larvae were operated on after the formation of the amniotic invagination, both meridional and transverse halves metamorphosed, and the percentage of metamorphosis increased as the stage of operation advanced. This result is probably due to the specific developmental mode of *Peronella*. Since the echinus rudiment of this form is found in the center of the body and develops to a large size, bisection in any direction will give each half approximately half of the rudiment, and the size of the half-echinus rudiment which goes to each half-larva becomes larger and larger with advance of the stage of operation. Consequently, regulation for metamorphosis of half-larvae will become increasingly easier as the operational stage advances. It seems quite possible that this central position and marked development of the echinus rudiment in the pluteus stage constitute the factor which takes primary importance, over that of the animal-vegetal axis, in determining the metamorphosing capacity of larval regions.

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#### SUMMARY

1. In the larva of the sand dollar *Peronella*, the mouth does not open, and no functional digestive tract is formed so that no feeding is necessary before metamorphosis.
2. Both the amniotic cavity and the hydrocoel take a median position in the larval body.
3. Cutting experiments show that single blastomeres of the two- or four-cell stage are totipotent regarding metamorphosing capacity.
4. Any meridional half of the larval stages has the capacity for metamorphosis.
5. The vegetal half of the sixteen-cell stage metamorphoses, but the animal half develops only to the blastula stage.



6. The vegetal halves, but not the animal halves, of the blastula and gastrula stages are able to metamorphose.

7. Both anterior and posterior halves of the pluteus stage are able to metamorphose.

8. The percentages of metamorphosis of partial larvae fall to a minimum at the gastrula-stages after which they rise while the regulative capacity falls as a course of a steady decrease.

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